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Gravitropism of the primary root of maize: a complex pattern of differential cellular growth in the cortex independent of the microtubular cytoskeleton

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Abstract. The spatio-temporal sequence of cellular growth within the post-mitotic inner and outer cortical tissue of the apex of the primary root of maize (*Zea mays* L.) was investigated during its orthogravitropic response. In the early phase (0–30 min) of the graviresponse there was a strong inhibition of cell lengthening in the outer cortex at the lower side of the root, whereas lengthening was only slightly impaired in the outer cortex at the upper side. Initially, inhibition of differential cell lengthening was less pronounced in the inner cortex indicating that tissue tensions which, in these circumstances, inevitably develop at the outer-inner cortex interface, might help to drive the onset of the root bending. At later stages of the graviresponse (60 min), when a root curvature had already developed, cells of the inner cortex then exhibited a prominent cell length differential between upper and lower sides, whereas the outer cortex cells had re-established similar lengths. Again, tissue tensions associated with the different patterns of cellular behaviour in the inner and outer cortex tissues, could be of relevance in terminating the root bending. The perception of gravity and the complex tissue-specific growth responses both proceeded normally in roots which were rendered devoid of microtubules by colchicine and oryzalin treatments. The lack of involvement of microtubules in the graviresponse was supported by several other lines of evidence. For instance, although taxol stabilized the cortical microtubules and prevented their re-orientation in post-mitotic cortical cells located at the lower side of gravistimulated roots, root bending developed normally. In contrast, when gravistimulated roots were physically prevented from bending, re-oriented arrays of cortical microtubules were seen in all post-mitotic cortical cells, irrespective of their position within the root.

Key words: Cell elongation – Cell shape – Gravitropism – Microtubule – Root growth – *Zea*

Introduction

Since the late 1920s, many concepts relating to plant tropisms have been influenced by the Cholodny-Went (CW) theory (Cholodny 1928; Went and Thimann 1937; Briggs 1992) in which a differential distribution of auxin is considered crucial for the differential flank growth of the organ in question. The CW theory predicts that gravity initiates a redistribution of auxin from the upper to the lower part of a horizontally positioned elongating region. Because plant roots generally have a characteristically high sensitivity to endogenous auxin, the level of which is close to, or slightly above, the optimum level for the maintenance of their growth (Pilet and Saugy 1985), even a small increase in auxin content elicits rapid growth inhibition (Gougler and Evans 1981; Ishikawa and Evans 1993). These features fit well with the CW theory. However, two recent findings are at variance with such a direct role for auxin, at least with respect to the initiation of root curvature. First, whereas root bending starts soon after the onset of gravistimulation – 10 min being sufficient to record a significant bending of maize roots (Nantawisarakul and Newman 1992) – it takes about 1 h to accomplish an auxin asymmetry in the root cap (Young et al. 1990); this asymmetry still needs to be relayed to the post-mitotic growth regions as it is here that differential cell growth drives root curvature (Shen-Miller et al. 1978; Barlow and Rathfelder 1985; Selker and Sievers 1987; Zieschang and Sievers 1991; Ishikawa and Evans 1991). Second, Ishikawa and Evans (1993) showed that gravitropism occurred even after inhibition of auxin transport in non-growing roots of maize, thus seemingly precluding redistribution of endogenous auxin as a factor causing differential growth. Similar findings were also reported for tomato roots (Muday and Haworth 1994).

Abbreviations: CMTs = cortical microtubules; CW = Cholodny-Went; FF = form factor; MT = microtubule

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Recent detailed studies on the root surface showed that the graviresponse starts simultaneously throughout the major part of the root growth region (Ishikawa and Evans 1991, 1994). A similarly coherent response in this region was also observed in the patterns of electrical and ionic events (Iwabuchi et al. 1989; Collings et al. 1992; Zieschang et al. 1993). This correspondence between the details of root growth and electric events indicates that the tissues controlling the graviresponse are distributed throughout the growing root apex. Presently, we do not know which tissue is particularly relevant for the control of root growth (Barlow 1982, 1989; Pritchard 1994). The epidermis has been indicated not to play the decisive role (Barlow 1982; Björkmann and Cleland 1991).

There is relatively little quantitative anatomical and cytological information on the temporal and spatial aspects of root graviresponses at the tissue and cellular levels (but see Shen-Miller et al. 1978; Barlow and Hofer 1982; Darbelley and Perbal 1984). Furthermore, quite unknown is the role of the microtubular cytoskeleton in the root gravireaction, or in tropic responses generally (Nick et al. 1991). Although reoriented cortical microtubules (CMTs) were described in outer cortical cells of the lower part of gravitropic maize roots (Blancaflor and Hasenstein 1993), the significance of this finding is unclear, especially since re-arrangements of the CMTs were recorded only after root bending was almost completed. Moreover, similar CMT reorientations can be induced by physical stress, as shown in pressure-treated epidermal cells of *Lolium* leaves (Cleary and Hardham 1993) and in mechanically stressed epidermal cells of maize coleoptiles (Zandomeni and Schopfer 1994). Therefore, rearrangements of CMTs during the graviresponse (Blancaflor and Hasenstein 1993) might be viewed not as a causative agent of differential growth, but rather as a consequence of internal tissue stresses developed as the root bends. A more detailed study on this point indicated that the first reoriented CMTs arrays appeared only after root bending had already started (Blancaflor and Hasenstein 1995).

A few years ago, we commenced (Baluška et al. 1990) a series of studies analysing the patterns of cellular growth and the tissue-specific distributions of CMTs (e.g. Baluška et al. 1992, 1993a, b) within the maize root apex. We have shown that the post-mitotic growth zone of the root apex is composed not of rapidly elongating cells only, but that there is a transitional, slower growing zone just distal to where rapid cell elongation takes place (Baluška et al. 1990). Cells located in this transitional zone show properties which might be relevant to the growth responses of gravistimulated maize roots (Baluška et al. 1994; Ishikawa and Evans 1992, 1993). In addition, several features of the cells of the outer cortex located in this transitional region (Baluška et al. 1993b) indicate that this could be a 'master' tissue involved in the regulation of root growth (Baluška et al. 1994).

Here, we present a spatio-temporal analysis of cellular growth in the post-mitotic growing portions of the maize root apex during its graviresponse, paying particular attention to any differences that there might be in the behaviour of the cells in the outer and inner cortex. These two groups of cells were earlier shown (Baluška et al. 1993b) to have differential responses to the plant hormone, ethylene. Also, in order to assess the possible involvement of CMTs in generating the gravicurvature, maize roots were treated prior to their gravistimulation with three anti-MT compounds known either to disassemble (colchicine, oryzalin) or to stabilize (taxol) the MT cytoskeleton, and their differential growth was then analysed.

Materials and methods

Plant material, growth conditions and gravistimulation. Caryopses of maize (*Zea mays* L. cv. LG 11), obtained from LG Semences S.A. (Chappes, France), were soaked for 6 h and germinated in moist rolls of filter paper at 22 ± 1 °C. Seedlings with straight, 2- to 3-mm primary roots were selected and transferred to small plastic growth chambers with a transparent side. Mounting of the intact seedlings within the chamber (40 per treatment) was done by gently pressing the grains into a wet block of Styrofoam so that the roots were free (i.e. not touching the surface of the Styrofoam) to grow vertically. Humidity inside the chamber was nearly saturated, a reliable indicator of which was the relatively high mean growth rate of the roots ($1.1\ mm \cdot h^{-1}$), a value comparable to that of roots growing in the wet paper rolls. Growth and gravitropism of roots took place in white light (neon tubes) with a photon fluence of $300\ \mu mol \cdot m^{-2} \cdot s^{-1}$ at the root surface. Temperatures in the growth chambers were maintained at 22 ± 1 °C. Roots were held in a vertical position during germination, as well as for the first hour after their transfer to the growth chamber. Then, in order to provide a gravistimulus, the growth chamber was gently rotated so that the roots were horizontal. Roots were photographed and their gravitropic bending estimated from prints as the angular deviation from horizontal of the apical 2-mm portion of tip. Statistical differences between mean values were assessed by Student's *t*-test.

Experimental treatments. To test the involvement of CMT arrays in gravitropism, vertical maize roots were exposed to various anti-MT agents (colchicine, oryzalin, taxol) immediately before gravistimulation. Treatment periods for colchicine (1 mM) and oryzalin (1 μM) were 2 h, as it had previously been shown that such treatments resulted in the complete disassembly of the MT cytoskeleton and that this disassembly state persisted for the duration of the experiment and that no swelling developed in this time (Baluška and Barlow 1993). Another group of roots was treated with taxol (0.1 mM) for 6 h in order to stabilize CMTs by lowering the critical concentration of tubulin required for MT assembly (Schiff et al. 1979).

Morphometrical microscopy. After 30, 60 and 120 min of gravistimulation, the apical 1-cm segments of primary roots were excised and fixed in FPA (formalin: propionic acid; 50% ethanol, 1:1:18, by vol.), and then embedded in paraffin wax. Median longitudinal sections, 10 μm thick, were prepared for anatomical and morphometric analyses (see Baluška et al. 1993a). Estimation of post-mitotic cell lengths and widths was performed using an ASBA image analyser (Wild, Heerbrugg, Switzerland). This image analyser also automatically computes from these data a Form Factor (FF) parameter, the value of which describes cell shape. Here, for example, FF = 6 corresponds to square, FF = 7 represents a rectangle with a side ratio (longitudinal: transversal) of 2:1, and FF = 12.4 corresponds to a rectangle with a side ratio (longitudinal: transversal) of 5:1.

Starting 1 mm behind the root-cap junction, cortical cell files were investigated in a 9-mm-long root segment which included the transitional region just behind the meristem (Baluška et al. 1990; Baluška et al. 1994) and the whole of the elongation zone. The transitional region of the maize root apex is an operationally defined zone where most cells are characterized by an approximately isodiametric shape (FF values approx. to 6) during the first phase of

post-mitotic growth (Baluška et al. 1990). The morphometric data so gathered were pooled for each experimental set of roots and were then automatically handled with a polynomial fitting method (Grapher Software Inc., Golden, Colo., USA) that computed the best-fitted curves of each variable with respect to distance from the root-cap junction.

Data from at least three roots per treatment were analysed. Two of the ten most-median sections were chosen to enable measurements of unbroken files of cells along the whole length of the growth region. Outer and inner cortical cells (Baluška et al. 1993b) were measured on both sides of the root in the chosen sections. About 1000 cells were measured in the inner cortex, and about 700 cells in the outer cortex of each experimental or control root at each time. Altogether, approx. 14 000 cells in the inner cortex and approx. 6000 cells in the outer cortex were automatically evaluated by image analysis. Differences between the parameters ascertained for the roots in each sample were found not to be appreciable.

Indirect immunofluorescence microscopy. After 30, 60 and 120 min of gravistimulation, 1-cm apical segments were excised and handled as described previously (Baluška et al. 1992). Briefly, longitudinal sections (5 μm thick) embedded in low-melting point Steedman's wax (m.p. 35 °C) were mounted on slides coated with Meyer's albumen. After digestion of cell walls with 1% hemicellulase, the median and neighbouring sections were incubated with a mouse monoclonal antibody raised against chick brain α-tubulin (Amersham International, Buckinghamshire, UK) diluted 1:200 with phosphate-buffered saline (PBS) for 60 min at 35 °C. Then, sections were stained with fluorescein isothiocyanate (FITC) conjugated anti-mouse IgG raised in goat (Sigma Chemical Co., St. Louis, Mo., USA) diluted 1:20 with PBS. After staining with toluidine blue (to quench autofluorescence) and mounting in anti-fade mountant (Baluška et al. 1992), microtubules were visualized in an Axiovert 405M inverted microscope (Zeiss, Oberkochen, Germany) equipped with epifluorescence and standard FITC exciter and barrier filters (BP 450-490, LP 520). Photographs were taken on Kodak T-Max film rated at 400 ASA.

Results

Time-course of cell length and cell shape changes during the first 120 min of gravistimulation

Root growth. Elongation rate of the control vertical roots was 1.06 ± 0.09 mm·h^{-1}. In the cases of pre-treatments with anti-MT compounds, the root growth rates of vertical roots slightly dropped during the next 2 h. The values were as follows: 0.89 ± 0.07 mm·h^{-1} for oryzalin, 0.90 ± 014 mm·h^{-1} for colchicine, and 0.85 ± 0.10 mm·h^{-1} for taxol. The mean angular deviations of the tips, due to gravitropism over a 2-h test period, were as follows: control $49.4 \pm 0.5°$, oryzalin $56.8 \pm 0.3°$, colchicine $56.3 \pm 0.5°$, taxol $57.1 \pm 0.5°$. The mean values from the three experimental treatments differed significantly ($P > 0.001$) from the control mean value, but did not differ amongst themselves. The main finding, nevertheless, is that the anti-MT treatments did not impair gravitropism.

Cell lengths. Cell length was a sensitive parameter that anticipated root bending throughout the course of the graviresponse (0–120 min). Cell width changed to a considerable lesser extent (data not shown). Although there was a general trend towards slightly greater cell widths at the lower side of gravistimulated roots, in the case of the outer cortex these changes were insignificant.

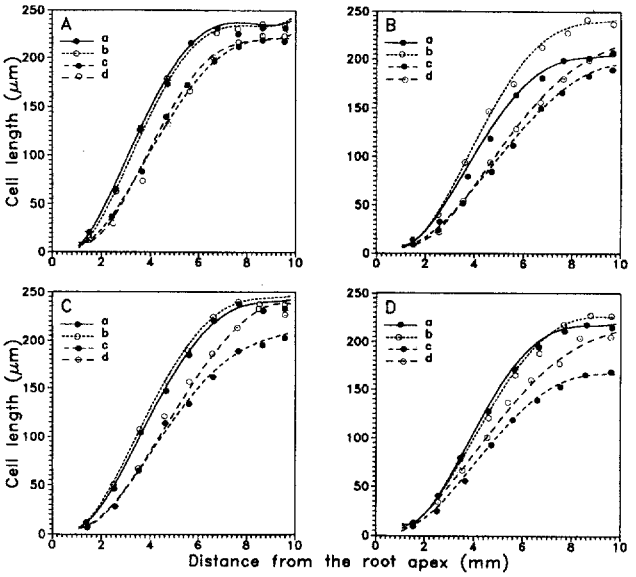

Fig. 1A–D. Best-fitted curves tracing the course of cell length changes in the outer cortex (*a*, *b*) and inner cortex (*c*, *d*) along the post-mitotic growth regions of gravistimulated maize roots (*a*, *c*, lower part; *b*, *d*, upper part). Periods of gravistimulation were: **A** 0 min (vertical control), **B** 30 min, **C** 60 min, **D** 120 min. Points indicating mean cell lengths for each 1 mm root segment are also included on the curves. However, these are given only as guides to the actual length dimensions of the cells. The curves were not fitted using these mean values; rather, the fits were made from the whole collection of cell length data, gathered continuously along the length of the root

In vertical control roots, mean final cell lengths for the outer and inner cortex cells were estimated as approx. 240 ± 13 μm and 220 ± 12 μm, respectively. After 30 min of gravistimulation, the final cortical cell lengths were, in most of the cell files inspected, less than the final control cell lengths. An exception was noted for the outer cortical cells in the upper side of gravistimulated roots (however, in the transition zone, slightly lower cell length values are also obvious) (Fig. 1A, B). At this early stage of the graviresponse, prominent differences in lengths were found between cells of the upper and lower sides of the outer cortex where the respective mean final cell lengths were estimated as approx. 240 ± 20 μm and 200 ± 15 μm (cf. Fig. 1B line a and Fig. 1B line b). The cells of the inner cortex showed less difference. The respective final cell lengths of upper and lower sides were approx. 210 ± 10 μm and 190 ± 8 μm (cf. Fig. 1B line c and Fig. 1B line d). Apparently, some 40 μm 'loss' of cell length occurs during the first 30 min of gravireaction in the outer cortical cells of the lower side. This cell length differential could be explained by a sudden cessation of cell growth at the lower side of re-oriented maize roots, perhaps associated with some moderate shrinkage, as was earlier reported by Ishikawa and Evans (1991).

At 60–120 min of gravistimulation the situation was reversed (Fig. 1C, D). Cells of the outer cortex showed less differences in their lengths between the upper and lower sides, the mean final cell lengths being estimated as approx. 220 ± 14 μm and 210 ± 15 μm, respectively (see lines a and b in Fig. 1D). In contrast, cells of the inner

cortex at the upper and lower root sides now exhibited a length differential where the respective mean final cell lengths were approx. 200 ± 19 μm and 160 ± 20 μm (see lines c and d in Fig. 1D).

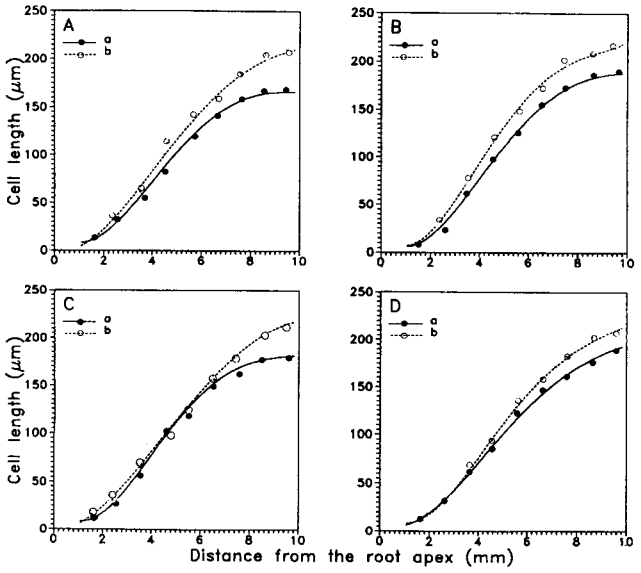

Fig. 2A–D. Best-fitted curves tracing the course of cell length changes in the inner cortex (*a*, lower part; *b*, upper part) along the post-mitotic growth regions of maize roots depleted of microtubules, determined after 120 min of gravistimulation. Roots were: **A** untreated (horizontal control), or were pre-treated with **B** taxol, **C** colchicine, **D** oryzalin. The same remarks about cell length and curve fitting as mentioned in the legend to Fig. 1A–D apply here also

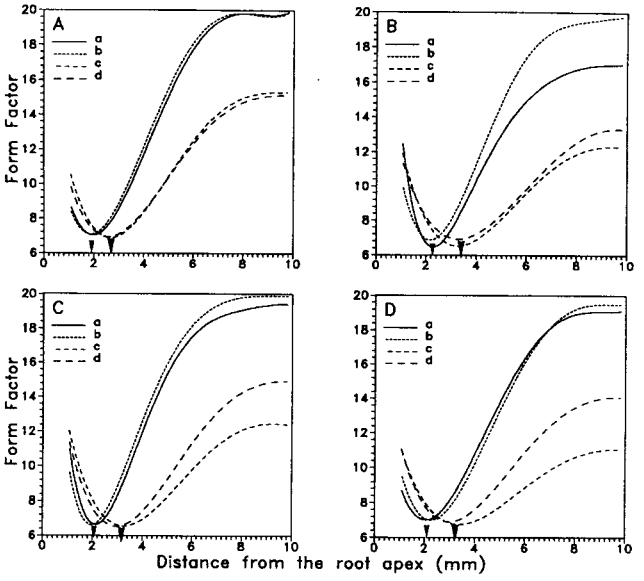

Fig. 3. Best-fitted curves tracing the course of cell shape (expressed as Form Factors) changes in the outer cortex (*a*, *b*) and the inner cortex (*c*, *d*) along the post-mitotic growth regions of gravistimulated maize roots (*a*, *c*, lower part; *b*, *d*, upper part). Periods of gravistimulation were: **A** 0 min (vertical control), **B** 30 min, **C** 60 min, **D** 120 min. The basal, proximal limit of the transitional region is indicated by *arrowheads* (small arrowheads for outer cortex, large arrowheads for inner cortex)

Comparison of the cell length distributions at all times (30, 60, 120 min) along the cortex of roots treated with all three anti-MT agents revealed insignificant differences (Fig. 2A–D) from those described above for untreated roots. Data for outer cortex are not shown. Such roots were still able to bend in response to gravistimulation (see Fig. 6A–H).

Cell shapes. The course of cell shape changes, as traced in the outer and inner cortex along the post-mitotic zones, emphasized the impact of gravistimulation on post-mitotic cellular growth. Cell shape, as represented by Form Factor (FF), reflects both the altered cell lengths and the slightly increased cell widths.

After 30 min in the horizontal orientation, the transitional growth region was shifted slightly basally (Fig. 3A–D), as indicated by the altered position of minimal FF values (small arrowheads for the outer cortex and large arrowheads for the inner cortex). Impaired longitudinal growth of most post-mitotic cortical cells was revealed by a decrease in FF values (compare Fig. 3A and 3B). The only exception was the cells of the outer cortex located in the upper part of graviresponding roots; these cells retained a final shape comparable to that of cells in control roots (line b in Fig. 3B). However, cell shapes in the upper side of the outer cortex are different from those in the lower side (the respective final FF values were approx. 20 and 17), whereas differences in the inner cortex are less (final FF values were approx. 13 and 12, respectively) at this early (30 min) stage of the gravitropic reaction. Final FF values of control roots were approx. 19.5 for the outer cortex and approx. 15 for the inner cortex.

A different situation was seen after 60 min of gravistimulation (Fig. 3C). A general tendency was for there to

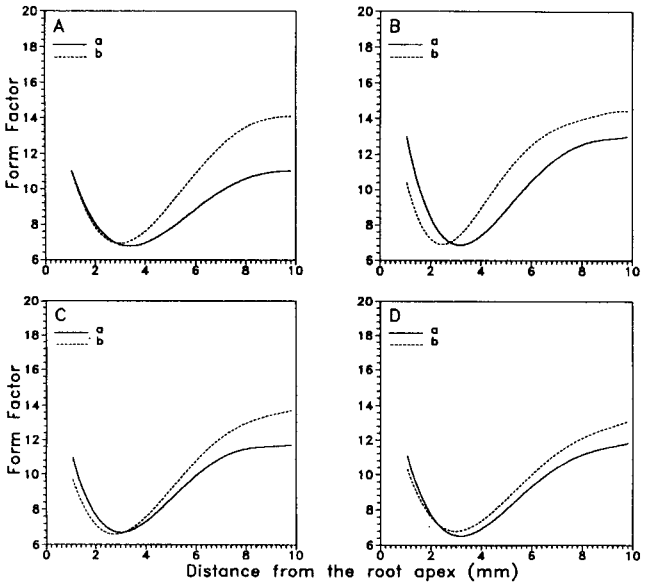

Fig. 4. Best-fitted curves tracing the course of cell shape (expressed as Form Factors) changes in the inner cortex (*a*, lower part; *b*, upper part) along the post-mitotic growth regions, determined after 120 min of gravistimulation. Roots were: **A** untreated (horizontal control), or were pre-treated with **B**, taxol, **C**, colchicine, **D**, oryzalin

be a partial recovery of the elongated cell shape (higher FF values). Recovery was least in the inner cortex at the lower side of the root (compare line c in Fig. 3C and 3B), but was more evident in the neighbouring outer cortex cells (compare line a Fig. 3C and 3B). This trend continued up to 120 min and, as a result, the initial differential was reversed (Fig. 3D). At this stage of the graviresponse, cells of the inner cortex showed greater differences in their shapes between the upper and lower side (the respective final FF values were approx. 15 and 11) than did the cells of the outer cortex (final FF values were approx. 19 and 18 in the upper and lower sides).

Following all three anti-MT treatments, cortical cells in the upper and lower parts showed differential FF values similar to those in control roots (Fig. 4A–D), although the first effects of the disturbed MT cytoskeleton on the cell growth polarity had started to be visible.

Organization of microtubules and root gravitropism after anti-microtubular pretreatments. Horizontal roots pretreated with either oryzalin (1 μM) or colchicine (1 mM) for 2 h were completely devoid of MTs throughout the growth zone, although diffuse fluorescence, due to unpolymerized tubulin molecules, picked out the cell walls, nuclei and cytoplasmic aggregations (Fig. 5K, L). Roots gravistimulated after taxol treatment contained well-ordered transverse bundles of CMTs in both their upper and lower sides (Fig. 5M). As all these roots bent normally after gravistimulation (Fig. 6A–H), it can be concluded that the mechanism which drives root bending

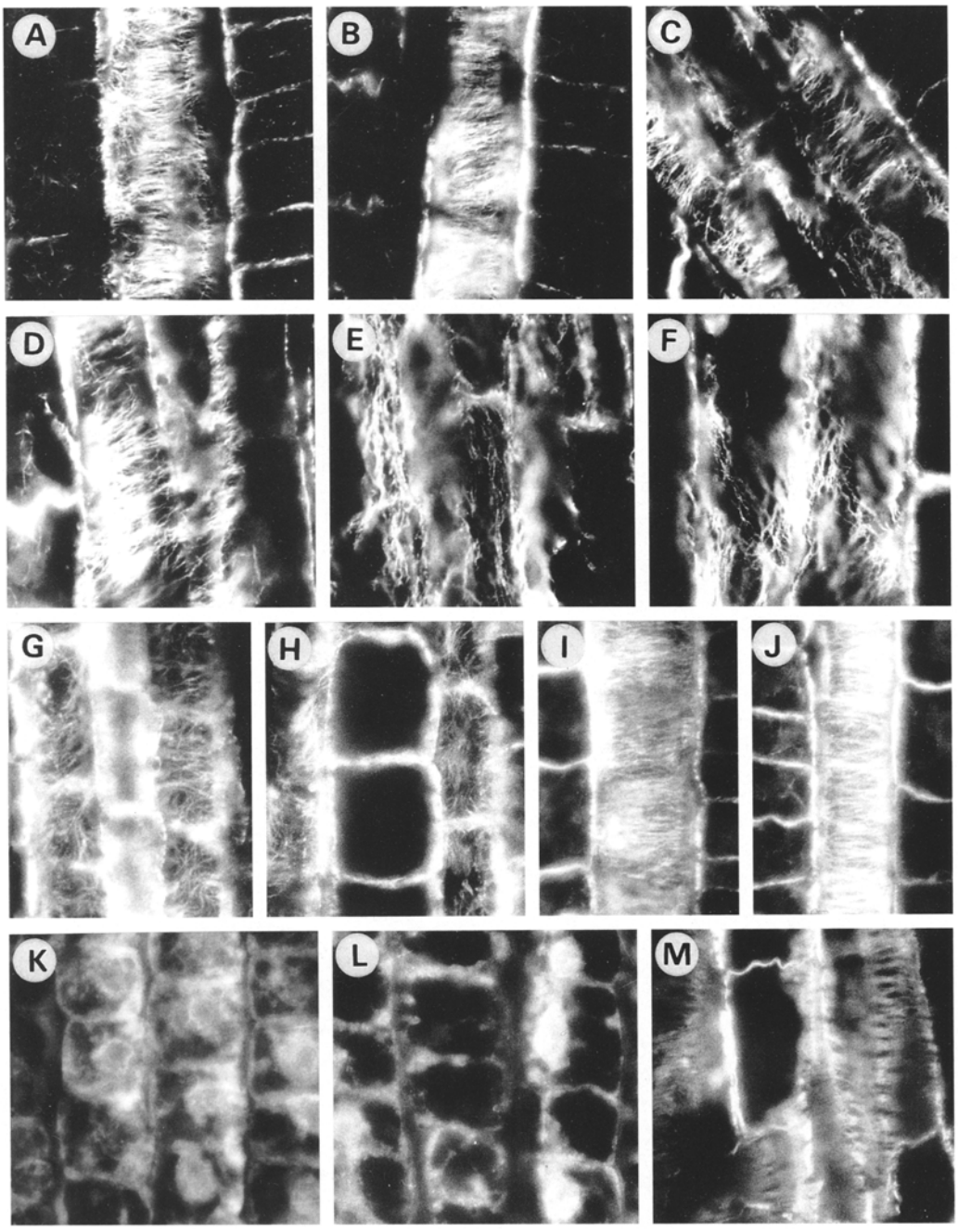

Fig. 5A–M. Distribution of CMTs in cortical cells of gravistimulated maize roots. When roots gravistimulated for 120 min were physically prevented from bending, a prominent disorientation of the CMT arrays (**A–F**) was seen in all cortical cells of the apex. **A–C** Early post-mitotic cells; **D–F** rapidly elongating cells. Affected CMT arrays were found in most cells of the *outer* cortex located in the *lower* part of roots gravistimulated for 60 min (**G**). At this time, disoriented CMT arrays were occasionally seen in cells of the *inner* cortex located at the *upper* part of gravistimulated roots (**H**). In contrast, most cells of the inner cortex (**I**), and all cells of the outer cortex, located in the upper part of gravistimulated roots, showed well organized transverse arrays of CMTs after either 60 min (**I**), or 120 min (**J**) of gravistimulation. Pre-treatment (2 h) of roots with oryzalin (**K**) or colchicine (**L**) disintegrated all MTs; in consequence, fluorescent spots of unpolymerized tubulin accumulated around the nuclei and along the cell walls. Taxol pre-treatment (6 h) resulted in stabilized bundles of CMTs (**M**) observed in both the upper and lower sides of gravistimulated roots

is apparently independent of the presence or absence of CMTs.

The lack of a relationship between CMTs and gravitropic bending suggests that the reorientation of CMT arrays from transverse to random or longitudinal, which often occurred in the outer cortex cells located in the lower part of graviresponding roots (Fig. 5G), may have been due to internal stresses elicited by the bending and, therefore, may not be a primary feature of the tropic response itself. The presence of similarly disoriented CMTs arrays in some cells of the inner cortex located in the upper part of graviresponding roots (Fig. 5H) supports the above notion. Physically preventing the development of curvatures in gravistimulated roots, by growing them for 120 min in horizontally oriented rolls of wet filter paper, resulted in the CMTs in cortical cells being disoriented or longitudinal throughout the post-mitotic zones in both the upper and lower sides of such roots (Fig. 5A–F). This supports the notion that the arrangement of CMTs was brought about by changes in the pattern of internal tissue stresses. Unchanged arrays of CMTs were found in most cortical cells located in the upper part of graviresponding roots (Fig. 5I–J).

Discussion

Data presented here on cellular aspects of the graviresponse of maize primary roots allow three conclusions to be reached. First, cell length differentials between upper and lower sides of the outer cortex seem to anticipate the onset of bending, which would imply that tissue tensions associated with the cells in this region are involved in the tropism. The outer cortical domain may thus represent a 'master' tissue involved in both the initiation and the cessation of the gravitropic bending. Second, inhibition of cell development (early postmitotic transition region) and premature maturation (elongation region) of inner cortical cells located at the lower side of the post-mitotic growth zones proved to be crucial for accomplishing the gravitropic bending. Third, the MT cytoskeleton plays no role in the development of the gravicurvature.

Detailed morphometric analysis enabled three phases of the graviresponse to be recognised at the cellular level. During the first 30 min of the response, cell elongation was interrupted temporarily in cortical cells throughout the post-mitotic growth region, the only exception being those cells in the outer cortex located in the upper part of

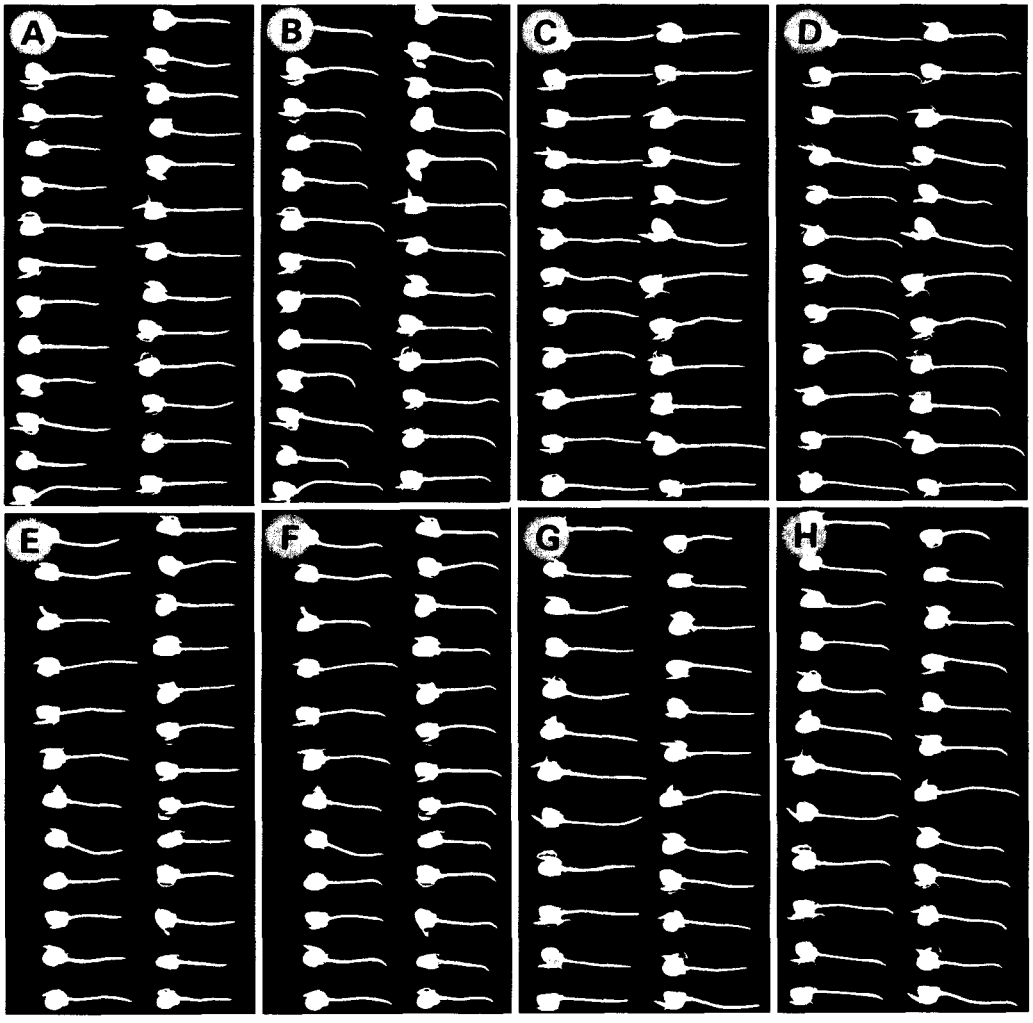

Fig. 6A–H. Growth and graviresponse of untreated control maize roots (**A, B**), and of roots exposed to either taxol (**C, D**), colchicine (**E, F**) or oryzalin (**G, H**). **A, C, E, G** Straight roots at the beginning of gravistimulation (0 min). **B, D, F, H** Root apices with appreciable bendings, which developed after 120 min of gravistimulation, irrespective of the anti-MT treatment

the roots. Then, during the next 30 min, a slight recovery of cell lengthening was accomplished, with the exception of inner cortex cells at the lower root side. Later, between 60 and 120 min, a third phase of the graviresponse was detected, in which cell elongation was impaired exclusively in the inner cortex. In this interval, the bending-related growth differential was developed in the inner cortex, which could be thus considered as a kind of 'motor' tissue in this morphogenetic event, due to inhibition of cell lengthening in the inner cortex of the lower root side.

What factors regulate the early gravity-induced growth response of the outer cortical cells in the upper and lower halves? With regard to the growth response of gravistimulated roots, auxin appears not to play a direct role, as predicted by the CW theory. This is because it takes about 60 min for the necessary asymmetry of endogenous auxin to develop in the graviresponding maize root apex (Young et al. 1990). Considering this, only the third phase (60–120 min) can be explained by the CW theory, as only at this time (2 h) was a significant increase of indole-3-acetic acid (IAA) recorded in the lower part of postmitotic growth regions in gravitropically bending maize roots (Saugy and Pilet 1984). Inhibition of postmitotic cell growth in the inner cortex at the lower root side might be directly mediated by a high endogenous level of auxin accumulating at the lower side of the gravistimulated root. Perhaps more relevant to the early growth response are the rapid changes of electric currents (Behrens et al. 1982; Iwabuchi et al. 1989; Collings et al. 1992) or of surface proton concentrations (Versel and Pilet 1986; Zieschang et al. 1993) recorded for growing root apices. Interestingly, the most prominent differentials between the upper and lower sides of gravistimulated roots in terms of these properties were in the transitional root region (for a review, see Baluška et al. 1994). This region is closer to the apex than the region of maximal relative cell elongation rate (Baluška et al. 1990; Zieschang and Sievers 1991; Zieschang et al. 1993), and it is here that the first signs of bending become visible (Barlow and Rathfelder 1985; Zieschang and Sievers 1991). A possible clue to interpret these findings might lie in the specific responses of the cells of this transitional region to internally or externally supplied auxin (Meuwly and Pilet 1991; Ishikawa and Evans 1993) and calcium (Ishikawa and Evans 1992), the two main effectors of the root graviresponse. Our morphometric data, showing a basipetal shift of the whole transition region, indicate that cells in this region were impaired in acquiring an elongated shape, especially when located at the lower root side. Moreover, cells located in the lower half of gravireacting lentil roots were delayed in their differentiation (Darbelley and Perbal 1984). All these observations suggest that differential elongation and development of early post-mitotic cells could be directly responsible for root bending.

Differences in cell lengths between cortical cells at the upper and lower root sides are, nevertheless, clearly evident throughout the elongation region. Although these are not associated with root bending during the early graviresponse, later the bending spreads basally, finally encompassing almost the whole elongation region (Ishikawa and Evans 1991; Masson 1995; see also Fig. 6). As cell length differentials in the main elongation region do not produce a root bending during the early graviresponse, they must contribute to tissue tensions within the growing apex. There are at least two reports supporting tissue tensions during the maize root graviresponse. First, Sack et al. (1990) confirmed numerous earlier observations of an unusual bulging of the upper side of the maize root apex just before the onset of the root bending. This phenomenon could be related to the decreased cell lengths in all cortical cells at the lower root side (Fig. 1A, B), preceding the formation of root curvature. Second, removal of the gravistimulus from bending maize roots rapidly causes a loss of gravicurvature, an effect termed 'springback' (Leopold and Wettlaufer 1989). This tonic response occurs regardless of the presence or absence of the root cap and indicates that discontinuation of the gravistimulation immediately allows inhibited postmitotic cells at the lower root side to recover their growth and, hence, straighten the root.

A striking feature of the first phase of the maize root graviresponse was a more prominent differential cell lengthening response of the outer cortical cells when compared to the inner cortical cells, indicating that the former tissue domain might control the elongation of the root apex as a whole. The cell length differential in the outer cortex clearly preceded the development of root bending and it cannot, therefore, be a simple geometric effect due to the curvature of the root. Rather, it might, through being a source of tissue tensions, initiate root bending. Later still, 120 min after the gravistimulus, root curvature was dissociated from the differences in cell lengths in the outer cortex of the upper and lower sides, because now the original equality of cell lengths had been re-established. New tissue tensions result at the outer-inner cortex interface and could, by acting in opposition, initiate the cessation of the bending process. In contrast to the outer cortex, a cell length differential (between the upper and lower root sides) in the inner cortex seemed invariably to follow the course of root bending. A plausible model for root growth control could, therefore, consist of two opposing cortical tissue domains in which the developmentally more advanced and potentially more rapidly growing cells of the outer cortex drive root growth, whereas the less advanced and potentially more slowly growing cells of the inner cortex (or the cortex-stele interface) (Barlow 1989; Pritchard 1994) restrict the root growth.

That CMTs are not involved in either the perception of gravity or the generation of root bending was unexpected in the light of generally accepted concept that the orientation of CMT arrays is a factor that controls cell growth polarity. However, this result could already be anticipated from the morphometric data which show that diminished cell elongation on the lower side of the root is not followed by a compensatory cell widening. Thus, during gravitropism, only cell lengthening is affected, while the direction of cellular growth is not altered. Similarly, we have evidence that CMT-independent root movement is also implicated in electrotropism of the maize root apex (data not shown). Another, but indirect, support comes from data relating to cell shapes in MT-deficient roots. These indicate that cells are capable, at least during the first 3 h after MT depolymerization, of

showing anisotropic growth comparable to those of control roots (data not shown). A similar finding was reported for oryzalin-treated *Arabidopsis* roots where normal elongation occurred, despite partial disintegration of the CMT arrays (Baskin et al. 1994). All this suggests that, even though CMTs may be destroyed, some 'memory' exists in the cells temporarily enabling them to continue anisotropic growth, thus precluding a direct role of CMTs in the short-term control of polarized cell growth. Similarly, Shibaoka (1994) reviewed several recent observations showing that reorientations of CMTs are not causally involved in phytohormone-induced short-term growth control.

The most direct support for the independence of the graviresponse from MTs was obtained from roots where gravicurvatures developed following MT disassembly by treatments with colchicine or oryzalin. A number of other indirect observations also support the MT-independent nature of the root graviresponse. For instance, after 60 min of gravistimulation, disorientation CMT arrays sometimes occurred in the inner cortical cells in the upper side of gravistimulated roots, and all post-mitotic cortical cells, irrespective of their position, exhibited reoriented arrays of CMTs when roots were physically prevented from bending. Furthermore, stabilization of MTs by taxol preserved their transverse (with respect to the cell axis) orientations in both halves of the gravibending root apices. This condition was also found to be compatible with gravitropism.

The reorientations of CMTs, as described in the outer cortical cells at the lower side of gravistimulated maize roots by Blancaflor and Hasenstein (1993), appear to be only the consequence of internal tissue stresses elicited during root bending. More recently, the onset of the root bending was reported to precede the reorientation of CMTs (Blancaflor and Hasenstein 1995). Similar re-distributions of CMTs have been described in plant cells after application of simple pressure treatments (Cleary and Hardham 1993), while the mechanical bending of maize coleoptile segments induced a massive transverse-to-longitudinal re-orientation of CMT arrays at the compressed (inner) side (Zandomeni and Schopfer 1994). Significantly, complete disintegration of the microtubular cytoskeleton was recently found not to affect cytoplasmic streaming and gravitropic growth in *Chara* rhizoids (Braun and Sievers 1994).

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